

## Peroxisome Proliferator-Activated Receptor (PPAR $\alpha$ ) Targeted Mutation Mouse Model

*Mice that lack a functional peroxisome proliferator-activated receptor gene exhibit several alterations in lipid metabolism that are expressed both constitutively and in response to peroxisome proliferators like hypolipidemia-inducing drugs, yet they are healthy and develop normally.*

### Applications for the PPAR $\alpha$ Targeted Mutation Mouse Model (model 001640)

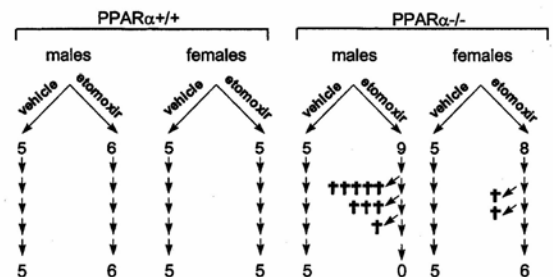
PPAR $\alpha$  Targeted Mutation Mice exhibit alterations of intracellular lipid processing, particularly in response to peroxisome proliferators. In wild-type mice, peroxisome proliferators are compounds that induce lipid catabolism and an associated intracellular increase in peroxisome number and enzymatic activity. These compounds form a diverse group that includes hypolipidemic drugs (fibrates), industrial chemicals (plasticizers, herbicides, chlorinated hydrocarbons) and certain endogenous fatty acids and steroids.

Peroxisome proliferator-activated receptors (PPAR) are proteins through which peroxisome proliferators interact with cells. PPAR proteins belong to the nuclear hormone receptor family, along with steroid hormone receptors. PPAR $\alpha$  in particular has been implicated as a key regulator of transcription of several genes, including those that encode lipid-metabolizing enzymes and the major apolipoproteins in HDL, apoA-I and apoA-II. PPAR $\alpha$  is thought to influence energy metabolism not only in the liver, but in heart, muscle, kidney, and adipose tissue. A role for this receptor in inflammation also is being elucidated.

PPAR $\alpha$  Targeted Mutation Mice exhibit alterations in several aspects of lipid storage and processing and show signs of altered inflammatory responses. Potential applications of the model include:

- Designing new drugs for treatment of hyperlipidemia
- Modeling human impairments of fatty acid processing
- Evaluating the influence of PPAR $\alpha$  on lipid processing in different cellular compartments (microsomal, mitochondrial, peroxisomal)
- Mapping tissue-specificity of PPAR $\alpha$ -dependent cellular activities
- Refining models of the induction of inflammatory mediators
- Exploring interrelationships among sex hormones and regulators of lipid balance

**A Gender-related Defect in Lipid Metabolism and Glucose Homeostasis in Peroxisome Proliferator-activated Receptor  $\alpha$ -deficient Mice**



Inhibition of transport of long chain fatty acids into mitochondria results in sex-influenced death in PPAR $\alpha$  Targeted Mutation Mice (PPAR $\alpha$ -/-). Numerals refer to number of mice in each group at beginning (top) and end (bottom) of experimental protocol. Each arrow denotes a single daily injection of vehicle or etomoxif. Each dagger symbol denotes death of a mouse.  
Djouadi, F., et al. (1998) *J Clin Invest*, Vol. 102, pp. 1083-1091.

## Features of PPAR $\alpha$ Targeted Mutation Mice

- Expression of an abnormal mRNA for PPAR $\alpha$ .<sup>1</sup>
- Absence of PPAR $\alpha$  protein.<sup>1</sup>
- Lack of classic responses to peroxisome proliferators (hepatomegaly, proliferation of peroxisomes in hepatocytes, lowering of serum lipoprotein and triglycerides).<sup>1,2</sup>
- Altered serum lipid profiles.<sup>3</sup>
- Over-expression of HDL lipoproteins, especially apoA-I.<sup>2</sup>
- Lack of induction of mRNAs for enzymes of lipid oxidation following exposure to peroxisome proliferators.<sup>1,4,5</sup>
- Intracellular build-up of lipids in liver<sup>1</sup> and gonadal adipose tissue<sup>3</sup> with exposure to peroxisome proliferators
- Gender-linked susceptibility to myocardial and hepatic lipid accumulation and fatal hypoglycemia after pharmacologic interruption of mitochondrial lipid utilization.<sup>6</sup>
- Partial protection from toxic effects of the peroxisome proliferator and commercial plasticizer di(2-ethylhexyl)phthalate DEHP.<sup>7</sup>
- Altered inflammatory response to endotoxin (*E coli* lipopolysaccharide).<sup>8</sup>
- Normal growth, weight, fertility and viability, with no observable developmental or anatomical defects.<sup>1,3</sup>

## Scientific Profile of PPAR $\alpha$ Targeted Mutation Mice

**A functional PPAR $\alpha$  protein is absent in PPAR $\alpha$  Targeted Mutation Mice.**<sup>1</sup> A mutated form of the mRNA for PPAR $\alpha$  is produced in knockout mice, which prevents expression of the protein. Liver cells, which normally produce quantifiable responses to administration of peroxisome proliferators, show no evidence of the PPAR $\alpha$  protein by Western blot analysis. This mutation has been bred onto both a C57BL/6N and a Sv/129 background. Many features are shared by both lines, though constitutive differences in serum cholesterol and triglycerides have been reported between the two.<sup>3</sup> Taconic's PPAR $\alpha$  Targeted Mutation are carried on C57BL/6 N inbred strain.

**Liver responses to peroxisome proliferators are abolished in PPAR $\alpha$  Targeted Mutation Mice.**<sup>1</sup> Wild mice respond to administration of peroxisome proliferators (e.g., clofibrate, Wy-14,643) by liver hepatomegaly (both hypertrophy and hyperplasia of hepatocytes are observed). This response is detectable in wild-type mice by an increase in liver weights of up to three times that of controls after two weeks of exposure to these chemicals. By contrast, PPAR $\alpha$  Targeted Mutation Mice maintain normal liver weights.<sup>1,8</sup>

**On the ultrastructural level, peroxisome proliferation is not induced in hepatocytes of knockout mice.**<sup>1</sup> This is in marked contrast to the response seen in wild-type mice, in which exposure to clofibrate or Wy-14,643 causes increases in the number, size, and staining intensities of peroxisomes, as well as conspicuous swelling of mitochondria. A unique observation in hepatocytes of PPAR $\alpha$  Targeted Mutation Mice is abundant accumulation of lipid droplets in response to peroxisome proliferators. These ultrastructural observations indicate that intracellular processing of lipids, at least in response to exogenous triggers of lipid metabolism, is aberrant in the knockout mice.

**Constitutive expression of lipid-metabolizing genes is significantly lower in hepatocytes of knockout mice.**<sup>3,4</sup> Compared to wild-type mice, the activity of several mitochondrial enzymes involved in  $\beta$ -oxidation of long-chain fatty acids is reduced. Interestingly, expression of microsomal and peroxisomal enzymes is similar to that of wild-type mice, indicating that PPAR $\alpha$  selectively influences enzyme activities. These observations combined explain the overall increase in lipid accumulation within hepatocytes.

**Induction of several lipid-metabolizing enzymes by peroxisome proliferators is abolished in PPAR $\alpha$  Targeted Mutation Mice.**<sup>1,4,5</sup> Administration of the prototypical peroxisome proliferators, fibrates (Wy-14,643) and clofibrate, induces expression of the mRNAs for several lipid-oxidizing genes in wild-type mice. Analyses of the activities of many of these enzymes in knockout mice showed no induction by these drugs<sup>1,4</sup> or by trichloroethylene,<sup>5</sup> except for a slight response

of peroxisomal thiolase.<sup>1</sup> These findings suggest that PPAR $\alpha$  is an important mediator, at the gene expression level, of peroxisome proliferator-induced lipid oxidation.

**Inactivation of the PPAR $\alpha$  gene alters serum cholesterol and/or triglyceride levels.** Earlier generations of the PPAR $\alpha$  Targeted Mutation Mice, which had a mixed C57BL/6N x Sv/129 background, exhibited elevated serum cholesterol and HDL cholesterol, but not triglycerides.<sup>1</sup> More recently, a study of mice in which the knockout gene was established separately on inbred Sv/129 and C57BL/6N backgrounds<sup>1</sup> reported elevated triglycerides but not serum cholesterol in the C57BL/6N knockout mice (Taconic's line). The authors comment that the C57BL/6N line is more appropriate for studies of atherosclerosis, however, because it forms atherosclerotic plaques on a high-fat diet.

**Interruption of lipid transport into mitochondria is always fatal in male knockout mice but only in a fourth of female mice.** In an experiment that pharmacologically blocked transport of long-chain fatty acids into mitochondria, knockout mice were unable to adapt as do wild-type mice, by up-regulation of PPAR $\alpha$  protein expression and its activation of non-mitochondrial oxidation pathways.<sup>6</sup> Instead, lipids accumulated in tissues that typically rely on fatty acid metabolism for energy (heart and liver), and hypoglycemia resulted as glycogen reserves became depleted. All male knockout mice died as a result of hypoglycemia, most within 24 hours. Interestingly, only a fourth of female knockout mice died, and those that survived showed little histological evidence of cellular lipid accumulation. Unlike males, females experienced a rebound in serum glucose after an initial hypoglycemia. These data implicate a role for estrogen in tapping alternate, non PPAR $\alpha$ -dependent catabolic pathways under conditions of energy stress. This was strongly supported by experiments in which male mice treated with estrogen survived their drug-induced hypoglycemia and showed a female-like rebound in serum glucose levels.<sup>6</sup>

**PPAR $\alpha$  Targeted Mutation Mice respond differently in an experimental model of**

**induced inflammatory response.** In wild type mice, exposure to LPS (lipopolysaccharide, an endotoxin derived from *E. coli*) stimulates a release into the bloodstream of tumor necrosis factor (TNF), a potent inflammatory mediator produced by macrophages. This response was increased fivefold in a study that combined LPS exposure with fenofibrate, a peroxisome proliferator.<sup>8</sup> While there was not a significant difference between control-fed wild-type or knockout mice in terms of the TNF-release response to LPS, the PPAR $\alpha$  Targeted Mutation mice responded to the combination of fenofibrate and LPS with a significant *reduction* in plasma TNF. These and other data in the same study led the authors to conclude that PPAR $\alpha$  plays a role in inflammatory mediation *in vivo* that may be direct or indirect and is part of a complex system. Taconic's PPAR $\alpha$  Targeted Mutation Mice can contribute to further elucidation of these mechanisms.

**Toxins that exert their effects through PPAR $\alpha$  may have reduced or no effect on knockout mice.** The peroxisome proliferator and commercial plasticizer, di(2-ethylhexyl)phthalate (DEHP) does not induce in knockout mice several of the toxic responses that it causes in wild-type mice, such as weight loss, liver lesions, and death.<sup>7</sup> The development of kidney and testicular toxicity in both groups of mice, however, indicates that non-PPAR $\alpha$ -dependent mechanisms mediate some of this toxin's effects.

**PPAR $\alpha$  Targeted Mutation Mice are fertile, healthy, of normal weight, and have no gross defects.** In spite of the several lipid metabolic alterations in these mice, they appear to undergo normal development and maintain adequate lipid homeostasis. Gonadal lipid stores are somewhat higher in both genders.<sup>3</sup>

## Origin of the Model

The PPAR $\alpha$  Targeted Mutation Mice from which Taconic's line is derived were developed by Frank Gonzalez and colleagues at the Laboratory of Metabolism, NCI, NIH. A disruption of the PPAR $\alpha$  was accomplished by gene targeting, using a vector that consisted of exon 8 of the gene (from a current BALB/c

mouse DNA library), in which 83 base pairs were deleted and replaced with a neomycin resistance cassette.<sup>1</sup> A herpes simplex thymidine kinase cassette and homologous sequences flanking the gene were also included in the construct. The vector was introduced into J1 ES cells derived from the 129S4 strain by electroporation.

Targeted cells from one clone were injected into C57BL/6N blastocysts at age 3-5 days and implanted in pseudopregnant mice. Five 129S4 x C57BL/6N chimeras were generated, of which three males were bred to C57BL/6N females. Their heterozygous offspring were mated to produce mice that were homozygous for the disrupted gene. Homozygotes were back-crossed to C57BL/6N mice for ten generations before they were received at Taconic and embryo transfer derived. The line was back-crossed to C57BL/6N for two additional generations and is currently maintained by mating male and female homozygous mice to produce homozygous offspring.

## Ready for Your Experiments

Taconic's PPAR $\alpha$  Targeted Mutation Mouse Model is maintained in Isolator Barrier Unit (IBU<sup>TM</sup>) facilities. Mice are shipped in Taconic Transport Cages (TTC<sup>TM</sup>) and come with an up-to-date health report documenting their Murine Pathogen Free (MPF<sup>TM</sup>) health status. Barrier housing conditions are recommended for maintenance of PPAR $\alpha$  Targeted Mutation Mice.

## Related Mouse Models from Taconic

Taconic provides a number of mouse models relevant to lipid transport and metabolism. Call or fax for information about these additional models:

- **APOE2 Targeted Replacement Mouse (model 001547)** – expressing the human apoE2 protein instead of murine apoE, with several abnormalities of lipid physiology, including elevated serum levels, altered lipoprotein profiles, and early development of atherosclerosis, all of which parallel features of human type III lipoproteinemia.

- **APOE3 Targeted Replacement Mouse (model 001548)** – expressing the human apoE3 protein instead of murine apoE, with normal serum cholesterol and triglyceride levels, but certain abnormalities of lipid physiology, including delayed clearance of lipoprotein particles (VLDL) and propensity to develop atherosclerosis on a high-fat diet.
- **APOE4 Targeted Replacement Mouse (model 001549)** – expressing the human apoE4 protein instead of murine apoE, with normal serum cholesterol and triglyceride levels but certain abnormalities of lipid physiology that are similar to those of ApoE3 Transgenic Mice; impairment in clearance of lipoprotein particles (VLDL) and development of atherosclerosis on a high-fat diet are more pronounced.
- **ApoB100 Microinjected Mouse (model 001004)** – develops atherosclerosis on a high fat/high cholesterol diet, due to expression of high levels of human apolipoprotein B100 and a resultant elevated plasma cholesterol (total and LDL-associated).
- **ApoE Targeted Mutation Mouse (model APOE)** – lacking the apoE gene, with highly altered lipid transport and metabolism, causing elevated serum lipids and spontaneous atherosclerosis that is further exacerbated by a high-fat diet.
- **CETP Microinjected Mouse (model 001003)** – expressing human cholesteryl ester transfer protein, a plasma enzyme normally absent in mice, which in humans mediates HDL-cholesterol transfer to VLDL and LDL and enhances cholesterol uptake into cells; transgenic mice have dramatically reduced HDL-cholesterol.
- **CETP-ApoB100 Double Microinjected Mouse (model 001007)** – develops atherosclerosis rapidly on a high-fat/high-cholesterol diet or normal mouse chow after six months, due to expression of both human CETP and apoB100 and consequent alterations in plasmalipid profile (elevated LDL-cholesterol, reduced HDL-cholesterol)

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## Taconic Transgenic Models Publication Reference List PPAR $\alpha$ Targeted Mutation Mice

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